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Attempt to Design Continuous Dissolution–Absorption System Using Everted Intestine Segment for In Vitro Absorption Studies of Slow Drug Release Formulations

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ABSTRACT

Reliable and predictive in vitro methods to quantify drug transport across the intestinal epithelium are required at an early stage in the drug development process for oral solid dosage forms. Also, the method should be useful for evaluating the effects of formulations on the transport. Several approaches have been used to obtain the most representative model of the intestinal epithelium. The everted and perfused intestine model presents many advantages relative to other methods. In this work, an attempt has been made to develop an in vitro continuous dissolution–absorption system to study the effect of slow drug release formulation variables on drug absorption. The dissolution studies were conducted on free drug and on two slow-release metformin hydrochloride (marketed) formulations. The studies yielded a dissolution–absorption relationship that can be used to predict dissolution or permeation-rate-limited absorption for two marketed formulations. The system also predicted permeation-rate-limited absorption for free metformin HCl, which is a highly permeable and aqueous soluble drug. Thus, the dissolution–absorption system may prove to be a valuable tool in formulation development. Broader evaluation of such a system is warranted.

INTRODUCTION

A major objective of the pharmaceutical industry is to develop drugs with good oral bioavailability. Administration of drug as a solid or liquid unit provides more convenient dosing and better patient compliance than painful and inconvenient iv dosage regimens. Good oral bioavailability occurs when the drug has maximum permeability and maximum solubility at the site of absorption (*1*). The extent of absorption of drug in vivo, thus, could be predicted based on permeability and solubility measurements (*2*). Hence, the intestinal permeability represents one essential part in the prediction of oral bioavailability (*3*). The intestinal permeability data can be used in preformulation studies to evaluate the effects of various pharmaceutical excipients on drug absorption (*4*).

A number of in vitro methods for assessing the intestinal permeability of a given drug have been developed and reviewed recently (*5*). In the last decade, the use of Caco-2 cell monolayers has gained popularity as an in vitro absorption screening tool. Nevertheless, the Caco-2 cell-based assay has some disadvantages. It is labor intensive, time consuming, has a high cost per assay, and requires a 21-day growth period and regular maintenance feeding for the preparation of stable monolayers.

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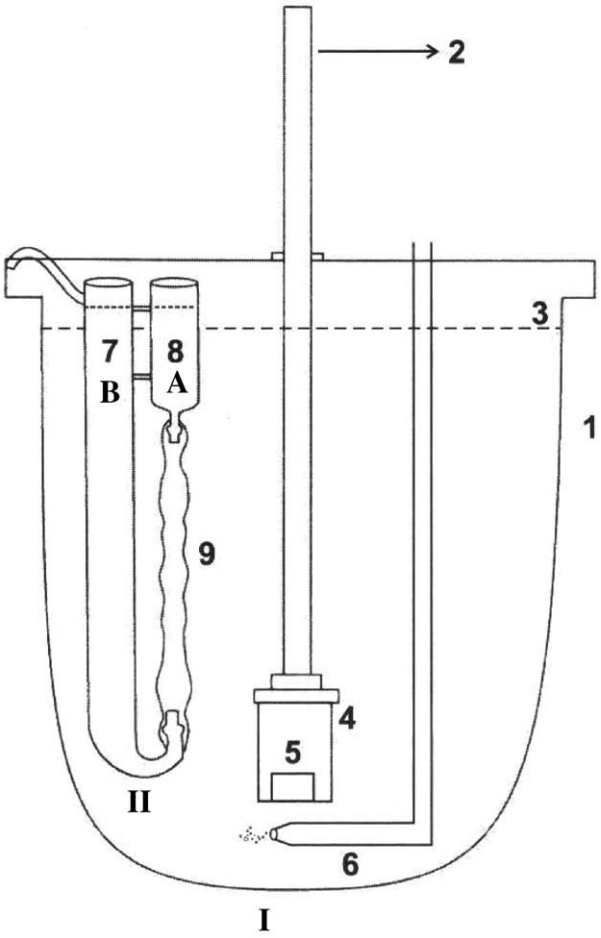
An alternative to the use of cultured cells is an in vitro absorption procedure based on in situ perfusion and isolated sacs from animal intestines (*5*). One of the major drawbacks of the in situ perfusion method is that it is unsuitable for screening a large number of compounds.

In vitro absorption (permeability) studies based on isolated intestinal sacs are routinely performed. The advantages of this model are that it contains all the types of cells and mucus layer, is relatively fast and inexpensive, and can be used for preformulation studies (*5*). This kind of model is suitable for measuring kinetic parameters with high reliability and reproducibility (*6*). Several animal species including rat, rabbit, pig, dog, and monkey have been used in permeability studies based on isolated intestinal sacs (*7*). To the best of our knowledge, in vitro absorption studies using chicken intestine have never been reported. The chicken small intestine could be a useful model (*8*) for intestinal absorption based on the assumption that membrane permeability of drugs is not species-dependant, since the composition of plasma membrane of intestinal epithelial cells is similar across the species. Thus the permeability across the chicken intestinal segment could be expected to be the same.

The aim of the current work was two-fold: to develop an in vitro continuous dissolution–absorption system using the chicken intestinal sac to predict a dissolution–absorption relationship for



1. Dissolution flask
2. Rotating shaft
3. Dissolution medium
4. Basket
5. Tablet
6. Oxygen tube
7. Tube B
8. Tube A
9. Everted intestine

I. Dissolution–absorption system
II. Absorption (perfusion) apparatus

Figure 1. Design of continuous dissolution–absorption using isolated everted intestine system.

slow-release formulations and to test the sensitivity of the developed system to formulation variables. Furthermore, the model would have advantages as less labor intensive, less time consuming, lower cost per assay and, since slaughtered chicken is used, no special permission from animal ethical committees would be required.

In this research work, metformin HCl was selected as the model drug. Metformin HCl was selected because it is mainly absorbed in the upper intestinal lumen, 60–70% drug is absorbed unchanged, it is a highly permeable drug, and it is formulated in a slow-release oral dosage form.

MATERIALS AND METHODS

Materials

The drug metformin HCl was obtained as a gift sample from Warner Labs, Secunderabad (India). All other chemicals used were of analytical grade.

Slow-Release Tablet

Two marketed, slow-release preparations of metformin HCl, MRKT-I (USV, Mumbai) and MRKT-II (Torrent Research Lab, Ahmedabad), were used. Both formulations contained 500 mg of metformin HCl.

In Vitro Dissolution Studies of Metformin HCl Tablet (MRKT-I and MRKT-II)

The in vitro dissolution profile of each formulation was determined in USP dissolution Apparatus 1. The test was carried out in 1000 mL of distilled water maintained at 37 °C at a basket rotation speed of 75 rpm. Samples (5 mL) were withdrawn at pre-selected time intervals up to 2 hours, and the volume was replaced by distilled water after each withdrawal. The samples were analyzed for metformin HCl by UV absorbance at 234 nm using a UV spectrophotometer (UV-2401, Shimadzu, Japan). Percent drug release versus time was calculated (PCP-Disso-V3, Pune), and the mean of three determinations was used in the data analysis.

Isolation of Everted Intestine

Male white Leghorn chicks weighing between 500 and 600 g were bought from the local market. The Krebs–Ringer solution was prepared by combining 6.3 g NaCl, 0.35 g KCl, 0.14 g $CaCl_2$, 0.16 g KH_2PO_4, 0.15 g $MgSO_4 \cdot 7\ H_2O$, 2.1 g $NaHCO_3$, and 5 g glucose in one liter of distilled water.

For isolation of everted intestine, the chicks were slaughtered, a median incision of the abdomen was performed, and the small intestine was freed. The lumen was carefully cleared from mucus by rinsing with a pH 7.4 buffer solution (Krebs–Ringer solution). An intestinal segment of the first 6-cm length was removed and transferred to oxygenated Krebs–Ringer solution. It was washed thoroughly with

warm Krebs–Ringer solution. The proximal extremity of the intestine was turned back and ligated on a glass rod to form an everted bag.

Design of Continuous Dissolution–Absorption System Using Everted Intestine Segment

The in vitro continuous dissolution–absorption system design is illustrated in Figure 1-I. The system consisted of USP dissolution Apparatus 1 and a side-by-side perfusion apparatus holding isolated everted intestine segment (Figure 1-II). In this system, drug dissolution from the slow-release tablet and permeation across everted intestine occurred simultaneously. The dissolution medium used was 1000 mL of distilled water maintained at 37 ± 0.5 °C. The perfusion apparatus consisted of two glass tubes, A and B, connected together (Figure 1-II). Tube B had a bent cannula at its lower end, and tube A, a straight cannula at its lower end. The distance between the two cannula was kept constant. The isolated everted intestinal segment was fixed between the ends of tubes A and B as shown in the Figure 1-II. The ends of the intestine were tied in position with a thread. The apparatus was immersed completely into the dissolution vessel.

Procedure for Absorption Studies in the Continuous Dissolution–Absorption System

In the proposed design of a continuous dissolution–absorption system, sampling can be done simultaneously for measurement of the in vitro dissolution and absorption profiles of the drug. The dissolution–absorption studies were performed in two parts. In the first part of study, a marketed, slow-release tablet of metformin HCl was used. The dissolution medium consisted of 1000 mL distilled water maintained at 37 ± 0.5 °C. A fresh intestinal segment was clamped to the perfusion apparatus. The total volume of the absorption compartment (tube A and tube B of perfusion apparatus) was 35 mL of Krebs–Ringer solution. The drug diffused from dissolution medium (mucosal side) to the serosal side (absorption compartment). The marketed, slow-release tablet (MRKT-I and MRKT-II) was transferred to the dissolution basket of the designed system. The tablet was rotated at 75 rpm speed. Dissolution samples (2 mL) were withdrawn at preselected time interval up to 3 h. The dissolution samples were taken with replacement at 15, 30, 45, 60, 75, 90, 105, 120, and 180 minutes, and the released metformin HCl was determined spectrophotometrically at 234 nm. The transported drug from the absorption compartment was sampled with replacement (Krebs–Ringer solution) at 18, 33, 48, 63, 78, 93, 108, and 123 minutes and analyzed spectrophotometrically for transported metformin HCl at 234 nm. To allow time for drug to

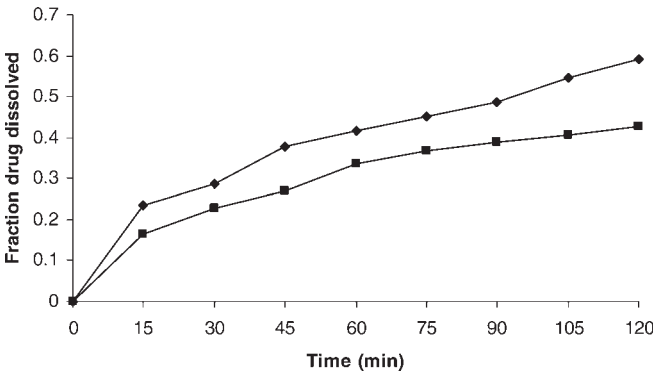


Figure 2. Fraction of drug dissolved vs time relationship for slow-release formulations of MRKT-I and MRKT-II formulation of metformin HCl from the dissolution–absorption system (♦ MRKT-I; ■ MRKT-II) (n =3).

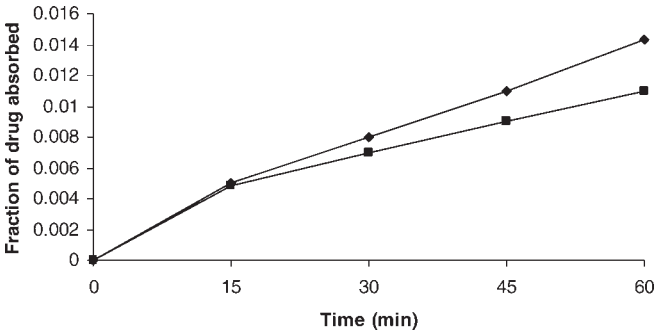


Figure 3. Fraction of drug absorbed vs time relationship for slow-release MRKT-I and MRKT-II formulation of metformin HCl from the dissolution–absorption system (♦ MRKT-I; ■ MRKT-II) (n =3).

circulate from the dissolution vessel to the everted intestine surface, absorption samples were collected 3 min later than their corresponding dissolution samples (*3*). The whole experiment was repeated in triplicate (n=3) using fresh dissolution medium as well as fresh intestinal segment each time.

The second part of the study was performed using four different concentrations (50 mg/L, 100 mg/L, 200 mg/L, and 500 mg/L) of powdered free-form metformin HCl. For each concentration, the dissolution medium was 1000 mL of distilled water maintained at 37 ± 0.5 °C. The paddle rotation speed was 75 rpm. Similarly, fresh intestinal segment was used for each concentration for the absorption study. The dissolved drug diffused from the mucosal to the serosal side and entered into the lumen of everted intestine (absorption compartment). The samples were collected for a total period of two hours. The absorption compartment was sampled with replacement (Krebs–Ringer solution) at 15, 30, 45, 60, 75, 90, 105, and 120 minutes, and metformin HCl was determined spectropho-

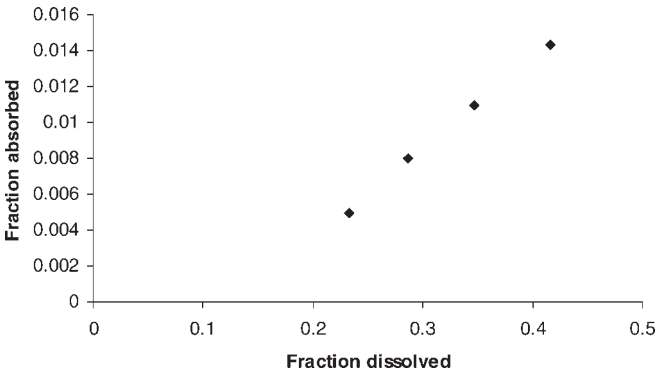


Figure 4a. F_a (fraction absorbed) vs F_d (fraction dissolved) relationship for slow-release MRKT-I formulation of metformin HCl from the dissolution–absorption system.

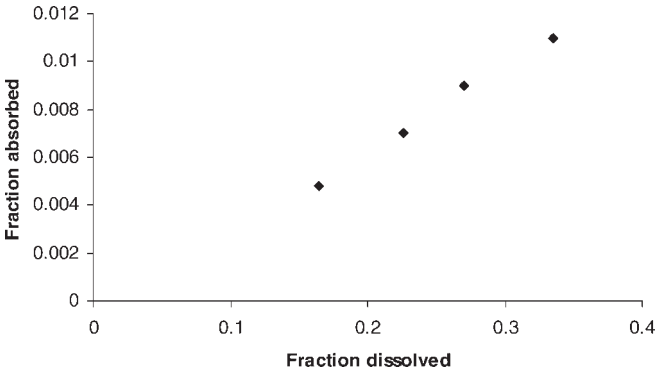


Figure 4b. F_a (fraction absorbed) vs F_d (fraction dissolved) relationship for slow-release MRKT-II formulation of metformin HCl from the dissolution–absorption system.

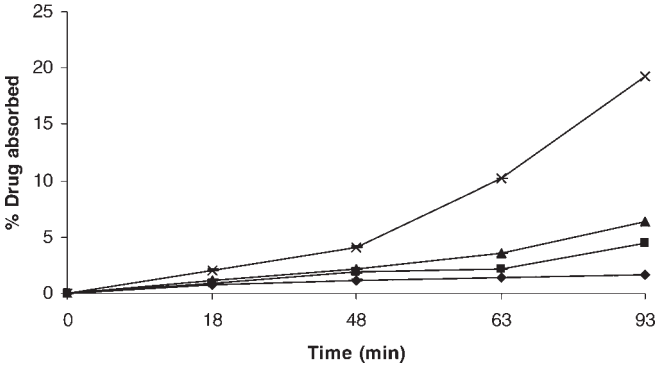
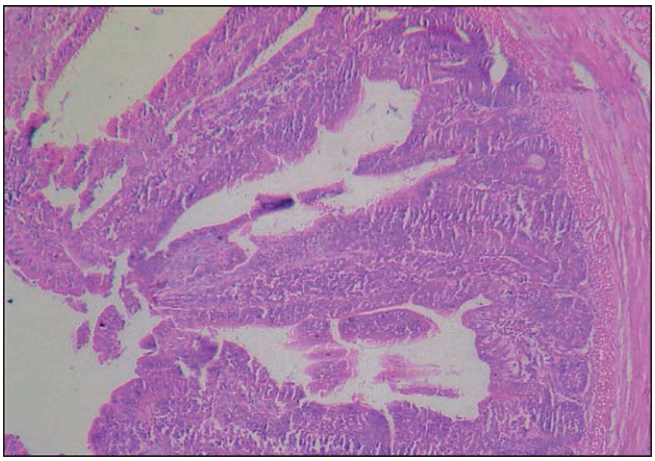
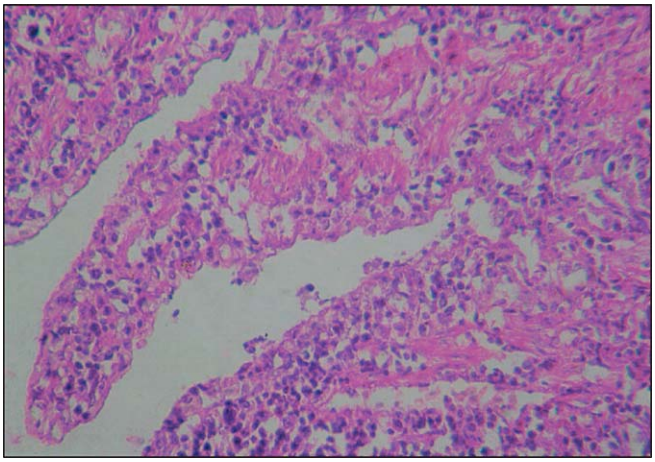


Figure 5. Percent drug absorbed vs time relationship of pure metformin HCl in different concentration levels from the dissolution–absorption system at identical time point (n =3)(♦ 500 mg/L; ■ 200 mg/L; ▲ 100 mg/L; × 50 mg/L).



(Photo 1)



(Photo 2)

Figure 6. Histological studies of chicken intestine. Photo 1 (10×) - at zero hour; Photo 2 (40×) - after two hours.

tometrically at 234 nm. The whole experiment was repeated in triplicate (n=3) using fresh dissolution medium as well as fresh intestinal segment each time.

Histopathology for Viability of Intestinal Cells

Pieces of intestine were flushed with normal saline at time zero and in 10% neutral buffered formalin at the end of the experiment (after 2 h) and processed by paraffin technique. Sections of 5-µm thickness were cut and stained with Haematoxylin-Eosin method (*9*).

RESULTS

The in vitro dissolution profiles of two marketed, slow-release tablets of metformin HCl are shown in Figure 2.

The objective of this study was to develop an in vitro continuous dissolution–absorption system using chicken

intestinal sac to predict a dissolution–absorption relationship for slow-release formulations. The dissolution–absorption plots were constructed by plotting the fraction of drug absorbed (F_a) against the fraction of drug dissolved (F_d). In order to construct a dissolution–absorption relationship from the continuous dissolution–absorption system, F_d versus time and F_a versus time were plotted as shown in Figures 2 and 3, respectively. While dissolution directly provides a profile of F_d versus time, the profile of F_a versus time from the above system is not obvious. That means the determination of the time for absorption includes time for dissolution (mean dissolution time) and time for intestinal permeation (*10*). Finally, the dissolution–absorption relationship was predicted at the same point by interpolating absorption data to dissolution sampling time of MRKT-I and MRKT-II formulations (Figures 4a and 4b, respectively). To further evaluate the ability of the system to predict properties of the oral solid dosage forms, the following equation was used:

$$F_a = 1/f_a \{1 - \alpha / \alpha - 1 (1 - F_d) + 1 / \alpha - 1 (1 - F_d)^{\alpha}\}$$

where F_a is the fraction of total amount of drug absorbed at time t, f_a is the fraction of the dose absorbed at $t = \infty$, α is the ratio of the apparent first-order permeation rate constant, and F_d is the fraction of dose dissolved at time t. The dimensionless parameter α reflects the degree to which dissolution limits overall drug absorption kinetics. An α value much greater than 1.0 indicates dissolution-rate-limited absorption. An α value much less than 1.0 indicates permeation-rate-limited absorption (*3*).

Also plotted in Figure 5 are the observed absorption studies of metformin HCl (powdered form) in dissolution medium at different concentration levels at identical time points. The viability of intestinal lumen cells was checked by histological studies. In Figure 6, Photo 1 (10×) is the slide of chicken intestinal epithelium just prior to experimentation. Similarly, Photo 2 (40×) is the slide of chicken intestinal epithelium immediately after completion of experimentation (period of 2.5 h).

DISCUSSION

The relationship between fractions of drug dissolved (F_d) versus time is shown in Figure 2 for both MRKT-I and MRKT-II slow-releasing tablets of metformin HCl (500 mg) (n = 3). From the graph, it is evident that there is a difference in the rate of dissolution of the two formulations, since $k = 0.0042$, $r^2 = 0.9973$ for MRKT-I and $k = 0.0032$, $r^2 = 0.9958$ for MRKT-II. This could be attributed to the difference in mechanism of drug release from the two formulations. During dissolution, no swelling was observed for the MRKT-I tablet that contained non-swellable, slow-release material. MRKT-II tablet showed swelling of polymer due to the presence of water-soluble, swellable polymer as release retardant.

The relationship between fractions of drug absorbed (F_a) versus time is shown in Figure 3 for MRKT-I and MRKT-II formulations. The graph indicates that the rate of absorption for MRKT-I ($k = 0.000231$, $r^2 = 0.9885$) is slightly faster than for the MRKT-II ($k = 0.000175$, $r^2 = 0.9562$) formulation. This implies the sensitivity of the continuous dissolution–absorption system.

The dissolution–absorption relationship resulting from the continuous dissolution–absorption system for the slow dissolving formulations of metformin HCl (MRKT-I and MRKT-II) are plotted in Figures 4a and 4b, respectively. The calculated α value was observed as 0.055 for MRKT-I and as 0.054 for MRKT-II. The metformin dissolution–absorption system predicts a permeation-rate-limited absorption for both slow-dissolving formulations, since the α value is much less than 1.0.

The absorption-versus-time relationship of free metformin HCl reveals that percent absorption increases with time. This is true for all concentrations of free metformin HCl in the dissolution medium as shown in Figure 5—less metformin, more absorption. Further, the observed profile for different concentrations of free metformin HCl at the same point exhibits a "reverse L" appearance. This is characteristic of permeation-rate-limited absorption (*3*). This is similar to the prediction of in vivo absorption mechanism made by Timmins et al. (*11*) for metformin HCl.

In histological findings, Photo 1 (Figure 6) reveals that intestinal villi have a normal pattern of epithelium with intact nucleus and sharp cytoplasm, indicating vital cells in the group of chicken intestinal epithelium. After 2 h of experimentation, it is observed that the epithelial cells of intestinal villi are enlarged and are headed with a different shape of vacuoles, indicative of vacuolar degenerative changes in the epithelium (Figure 6, Photo 2). The nuclei in almost all the cells were intact, indicating the vitality of the cells even after 2 h of experimentation.

Thus, it could be concluded that a successful attempt was made to design an in vitro continuous dissolution–absorption system using everted chicken intestine segments. However, to evaluate the usefulness of the chicken intestinal permeability model in biopharmaceutical classification system, further studies mentioned in the FDA guidelines are needed on the following points:

- Effect of drug solubility on absorption rate.
- Modifications in the absorption medium to increase or improve the functional viability of the membrane.
- Comparison of observed dissolution–absorption relationships of drug from clinical studies of that drug formulation.

➢ Comparison of Peff of different absorption compartments of chicken gastrointestinal tract.

REFERENCES

1. Trapani,G.; Iranco, M.; Trapani, A.; Lopedota, A.; Letrofa, A.; Galluci, E.; Micelli, S.; Liso,G. Frog intestinal sac: A new in vitro method for the assessment of intestinal permeability. *J. Pharm. Sci.* **2004**, *93* (12), 2909.
2. Egan, W.; Lauri, G. Prediction of intestinal permeability. *Adv. Drug Deliv. Rev.* **2002**, *54*, 273.
3. Ginski, M. G.; Polli, J. E. Prediction of dissolution–absorption relationship from a dissolution/Caco-2 system. *Int. J. Pharm.* **1999**, *177*, 117.
4. Saha, P.; Kou, J. H. Effect of solubilizing excipients on permeation of poorly water soluble compounds across Caco-2 cell monolayer. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 403.
5. Ferrec, E.; Christophe, C.; Artussen, P.; Brayden, D.; Fabre, G.; Gires, P.; Grillon, F.; Reuset, M.; Rubas, W.; Scarino, M. In vitro models of the intestinal barriers. *ATLA* **1999**, *29*, 649.
6. Leppert, P. S.; Fix, J. A. Use of everted intestinal rings for in vitro examination of oral absorption potential. *J. Pharm. Sci.* **1994**, *83*, 976.
7. Tukker, J. J. In Vitro Methods for the Assessment of Permeability. In *Oral Drug Absorption*; Dressman, J. B., Lennernas, H., Eds.; Marcel Dekker: New York, 2000; p 51.
8. Irvine, J. D.; Takahashi, L.; Lockhart, K.; Cheong, J.; Tolan, J. W.; Selick, H. E.; Grove, J. R. MDCK Cells: A tool for membrane permeability screening. *J. Pharm. Sci.* **1999**, *88*, 28.
9. Bancroft, J. D.; Cook, H. C. *Manual of Histological Techniques*; Chruchill Livingstone: New York, 1988; p 243.
10. Ingels, F.; Deferme, S.; Destexhe, E.; Oth, M.; Van den Mooter, G.; Augustijns, P. Simulated intestinal fluid as transport medium in the Caco-2 cell culture model. I*nt. J. Pharm.* **2002**, *232*, 183.
11. Timmins, P.; Dennis, A. B.; Vyas, K. A. Biphasic controlled release delivery system for high solubility pharmaceuticals and method. U.S. Patent 6,475,521, November 5, 2002.